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STUDIES IN ADSORPTION CHROMATOGRAPHY

I. THE CHROMATOGRAPHIC BEHAVIOR OF A HOMOLOGOUS SERIES OF ETHYL ESTERS

II. INVESTIGATION OF THE STERIC EFFECT OF ALKYL GROUPS ON THE ADSORPTION OF ANILINES

III. AN EVALUATION OF 2,4,7-TRINITROFLUORENONE AS A STREAK REAGENT

A THESIS

Presented to

the Faculty of the Graduate Division

by

Floyd Breland O'Neal

In Partial Fulfillment

of the Requirements for the Degree

Doctor of Philosophy in the School

of Chemistry

Georgia Institute of Technology

March, 1959

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Approved:

Date Approved by Chairman:

ACKNOWLEDGMENTS

The author wishes to express his gratitude to Dr. Jack K. Carlton for guidance, inspiration, and friendship. Dr. W. M. Spicer, Director of the School of Chemistry, and Dr. R. L. Sweigert, Dean of the Graduate Division, are among the many members of the staff of the Georgia Institute of Technology who have made this work possible. The Research Corporation and the National Institutes of Health have, by research fellowships, supported the major portion of this work. The author also wishes to express his gratitude to his wife for her patience and devotion during these school years.

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SUMMARY

PART I

The ratio of colorless to colored organic compounds is quite large. Since visual observations are employed so frequently in the laboratory, it would be extremely helpful to have a series of reagents to develop colors among the various organic compound classes for identification purposes. In adsorption chromatography, this identifying reagent could be applied directly to the column. The color of the zone produced and the relative location of the zone on the column would then be very useful in qualitative analysis. One compound class for which no suitable reagent has been reported in the literature is the large family of esters. It was decided to attempt to develop a reagent or series of reagents which would, under the proper conditions, produce a colored zone for identification of the esters on chromatographic adsorption columns.

Two reactions were considered for possible adaptation to adsorption columns. The first reaction involved the colored molybdate-xanthate complex. The reaction failed to give positive results under any conditions tried. The second reaction considered involved the colored hydroxamate-ferric ion complex. This reaction gave colored bands; reproducible results were obtained using Florisil as adsorbent but alumina and silicic acid failed in this respect.

The purple color of the hydroxamate-ferric ion complex occurred on the adsorption columns when a 0.01 M solution of the

ester in benzene is first streaked with the filtrate from a mixture of methanolic solutions of sodium hydroxide and hydroxylamine hydrochloric, then heated, and finally streaked with a perchloric acid solution of ferric perchlorate. The developing solvent was benzene. The heat source was an infrared lamp.

The R value, the ratio of the distance traveled by the ester to the distance traveled by the developing solvent, was recorded for each ester tested. Although several solutions of esters tested failed to give the purple color, the conclusion drawn was that the detection limit for these few compounds was not exceeded, since the pure compounds did give purple colors.

In general, it was noted that increased molecular weight in the side chain of the homologous series of ethyl esters did increase the R value. Substituents and branched side chains did affect the R value, although the exact nature of these effects is not obvious. It seems that chlorine atoms can reduce the electron density about the carbonyl oxygen, thus lowering its adsorption affinity. This effect was most notable with ethyl trichloroacetate, which gave an R value close to 1.00.

Detection of esters as the ferric hydroxamates can be easily performed when Florisil is used as the adsorbent. Separation of various esters was not attempted since no specific problem was at hand in this work. Work in this area in the future should include separation of various esters of similar properties, the work to include a study of the efficiency of recovery of the esters.

PART II

The conclusion has been advanced that the steric hindrance of the alkyl substituent on ortho-alkyl phenols is the most important influence of that group on the chromatographic adsorption of these compounds. This investigation of substituted anilines was undertaken in an attempt to elucidate the steric effect of alkyl groups on the adsorption properties of the amino group attached to an aromatic nucleus. Another purpose was to correlate the sizes of alkyl and non-alkyl groups, and, from this information and R values, deduce the steric interference of the non-alkyl group, as well as a relative value of other effects.

If the substituent is in the para position, no problem is encountered in the separation of steric effects from other effects. When the substituent is ortho to the amino group, this separation of various effects becomes more difficult. By a comparison of sizes and R values of several groups, one may be able to determine which effect is of major importance and if so, assign at least qualitative values to the various effects.

Various groups were studied in ortho and para positions. Methyl, ethyl isopropyl, and t-butyl groups constituted the alkyl series, while nitro, amino, and chloro groups constituted the non-alkyl groups. Some 2,6-disubstituted alkyl anilines were used, i.e., 2,6-dimethyl aniline, 2,6-diethylaniline, and 2,4,6-tri-t-butylaniline.

The anilines were chromatographed on both Florisil and silicic acid in various states of deactivation. The aniline solutions were

made up as 0.01 M solutions in benzene, and benzene was used as the developing solvent. The streak reagents employed for the detection of the anilines were ceric nitrate and p-nitrobenzenediazonium tetrafluoroborate. Ceric nitrate failed to produce a coloration with only two of the anilines used; p-nitrobenzenediazonium tetrafluoroborate did give a coloration in these two cases although it failed in several other cases.

Steric hindrance did seem to be the most important influence of the alkyl group on the adsorption properties of the o-alkyl anilines. The non-alkyl groups were found to generally exhibit noticeable other effects.

It was found by experimentation that silicic acid and Florisil could be brought to roughly the same state as to the adsorption properties of the anilines on their surfaces. Deactivation of the adsorbents was accomplished by drying, adding measured quantities of water, and mixing by a special method.

It has been demonstrated by this study that ortho- and para-substituted anilines may be efficiently separated on either of the adsorbents used by proper control of conditions. Two reagents for conjunctive use have been shown to be of value in the detection of aromatic amines.

Further investigations should be made to determine the actual efficiency of separation and recovery of o- and p-substituted anilines. Many other substituted anilines could be used in a similar study to determine more accurately if the conclusions drawn here need to be

modified. Another solvent system might be used in order to obtain substituted anilines in solution which are not soluble in benzene and hence were excluded from this study.

PART III

2,4,7-Trinitrofluorenone has been shown to be of great value in microscopic fusion work for the identification of many aromatic hydrocarbons and their derivatives. If this compound could be developed into a streak reagent capable of detecting a wide variety of aromatic nuclei, it would be valuable in a qualitative scheme of chromatographic adsorption analysis. Its greatest possible use would be in distinguishing between mono- and polynuclear aromatic compounds. Some reagents do this to some extent, but have no general color distinction among the various nuclei. It was thought worthwhile to determine if 2,4,7-trinitrofluorenone would possibly distinguish mononuclear aromatic compounds from polynuclear aromatic compounds.

The reagent was applied to many compounds adsorbed on Florisil and silicic acid, but was not found to be superior to the reagents already in use for detecting aromatic nuclei. Many of the compounds tested gave no reaction; more of the compounds tested did give visible reactions on Florisil than on silicic acid. 2,4,7-Trinitrofluorenone would be useful as a conjunctive streak reagent in those cases where the product with a particular compound is quite distinct and unique in color.

Some of the compounds required heat for the appearance of the colored zone, while others required no heat. In those cases where

heat was not required for the initial production of color, the color would become more intense with the application of heat. This suggests that the heating time might be lengthened and bring out colored zones in more cases, but extended heating causes the columns to crack and crumble, which result renders observations indeterminate.

PART I

THE CHROMATOGRAPHIC BEHAVIOR OF A HOMOLOGOUS SERIES OF ETHYL ESTERS

CHAPTER I

INTRODUCTION

The word chromatography originally referred to the process of producing a series of colored bands on a column of adsorbent by flushing a solution of natural pigments down the column. At the present time, the idea of color is not necessarily associated with chromatography. Perhaps the best definition of chromatography is that given by Strain (1): "Chromatography is the name given to a technique of analysis and/or separation in which there is a dynamic partition or distribution of dissolved or dispersed materials between two immiscible phases, one of which is moving past the other."

The fixed phase is the adsorbent. There is still no completely acceptable term for the mobile phase which is accurate for every type of chromatography. Excluding gas chromatography, the term developer is used in reference to the moving phase. The substance being removed by adsorption is referred to as the adsorptive.

In adsorption chromatography the fixed phase is a solid adsorbent, while in partition chromatography the fixed phase is a sorbed liquid phase. Applying the elution technique, a sample containing the adsorptive is placed on a packed column of adsorbent and then the developing solvent is added to the column to work the sample solution into the column. When the developing solvent front reached the bottom of the column, any excess developing solvent remaining on top of the

column is removed. Detection of the zone of adsorptive may then be carried out in one of several ways.

If the substance being chromatographed has a characteristic color of its own, observation alone will usually established the position of the zone. If the substance as adsorbed is colorless, one may follow any of the following procedures: (a) Use a second developer containing a substance which will react with the adsorbed substance to give a colored product which can be observed; (b) Extrude the packed adsorbent from the column and streak or spray onto the column a reagent which will give an observable product with the adsorptive; (c) Use ultraviolet light either with or without a streak or spray reagent for the detection of the adsorptive zone. Once the zone has been located, the distance from the top of the column to the zone front is measured, and the ratio of this distance to the distance the solvent has moved (the length of the column) is known as the R value (2).

Any theory of chromatography must of necessity be concerned primarily with the phenomenon of adsorption. Adsorption is not a recent discovery, as it was used commercially in the latter part of the eighteenth century in the wine industry. Adsorption is usually classified as either physical adsorption or chemisorption. The former consists of the formation of a condensed body of the adsorptive on the surface of the adsorbent, while the latter refers to a chemical reaction taking place between the adsorptive and adsorbent. When chemisorption occurs, the adsorptive is less easily removed by elution for recovery than when physical adsorption occurs.

Kipling and Peakall (3) have arrived at a series of criteria which they use as a basis for distinguishing between physical adsorption and chemisorption in a particular series of experiments. Prominent among these criteria is the observation that adsorption can be shown to occur at specific sites on the surface, as can sometimes be demonstrated by altering the surface. It follows from this statement that the amount of adsorptive chemisorbed can be related to the number of accessible sites on the surface of the adsorbent. The number of accessible sites will depend upon factors such as the physical condition of the adsorbent, the extent of hydration of the adsorbent, and the exact structure of the adsorbent surface.

Silicic acid can function as either a Lewis acid or a Lewis base. Elder and Springer (4) have assumed the surface structure of silica gel to contain both oxide and hydroxide groupings. Kipling and Peakall (3) have also arrived at this same conclusion from studies of silica gel. These latter workers proposed that the removal of water from silica gel (activation) increases the ratio of oxide to hydroxide groupings, this proposal being made when aliphatic alcohols were more strongly adsorbed on the activated silica gel. From these arguments, one may consider that hydrogen bonds may be formed either from the oxide oxygen atom (donor) on the adsorbent to a hydrogen atom (acceptor) on the adsorptive or from the hydroxide hydrogen atom of the adsorbent to some donor site on the adsorptive.

One of the goals in adsorption chromatography is to accumulate sufficient information to enable one to devise a systematic scheme of

rapid qualitative organic analysis requiring little time and effort. The equipment required for adsorption chromatography is neither complex nor expensive. Only short time periods are required to pack, develop, extrude, and streak the extruded column with a particular reagent or series of reagents.

Streak reagents are required to locate zones of colorless compounds, and at the same time they aid in identifying such compounds. If one possessed a series of streak reagents, one of which was specific for each class of compounds, these reagents, coupled with the chromatographic R values, would be a powerful tool in determining the compounds present in an organic mixture.

Streak reagents have been developed for many classes of compounds (5,6). While some of these reagents are not specific for certain compound classes, judicious use of several of the reagents will usually identify the class of compound present. A very common class of compound for which no streak reagent has been reported is the large family of esters. With so many reactions of esters listed in reference works, it was decided to attempt to find one reaction which would lead to a useful streak reagent for the esters.

Two reactions used by Feigl (7) for spot tests were considered for adaptation as a streak reagent. The first reaction considered involved the colored molybdate-xanthate complex (8). This reaction is intended to detect primary and secondary alcohols, but esters are partially saponified under the conditions of the test and hence might be detected. All alkali-alkyl xanthates form violet products with

molybdates in solutions containing an excess of mineral acid, the products being soluble in organic liquids. Unfortunately this test required streaking the column four times after extrusion from the chromatographic tube. Various orders of streaking with the different reagents gave no positive results.

The second spot test reaction considered involved the colored hydroxamate-ferric ion complex. Various combinations of the reagents listed by Feigl (9) with silicic acid and alumina as adsorbents produced no encouraging results. Goddu, LeBlanc, and Wright (10) had used a similar procedure for the quantitative estimation of esters spectrophotometrically. Using reagents quite similar to those of Goddu and coworkers, and an infrared lamp as a heat source, various adsorbents, developers, and streaking procedures were investigated. Reproducible results were obtained on Florisil but not on alumina or silicic acid.

The equations for the formation of the ferric hydroxamates are given below. The basic equations are those worked out by Feigl (9); the only changes made here are the use of perchloric acid and ferric perchlorate instead of hydrochloric acid and ferric chloride, respectively.

$$RCOOR_1 + NH_2OH + NaOH = RCO(NHONa) + R_1OH + H_2O$$

$$RCO(NHONa) + HClO_4 = RCO(NHOH) + NaClO_4$$

$$3\ RCO(NHOH) + Fe(ClO_4)_3 = 3\ HClO_4 + Fe[RCO(NHO)]_3$$

In an effort to evaluate the usefulness of the streak reagents used to locate the ester band, a homologous series of ethyl esters was chromatographed. The acid portions of the esters were normal saturated chains. This series was followed by a study of various esters which were readily available. From the homologous series one could determine if a certain chromatographic behavior pattern were evident, such as an increase in R value with chain length. With the more general esters of various kinds which were readily available, one could determine if the streak reagents developed were useful for detecting esters as a compound class. The work indicated that esters were detectable on Florisil columns as ferric hydroxamates.

It is desirable in adsorption chromatography to have R values of less than 0.50 or 0.60, since R values greater than these are difficult to duplicate. One would also desire some separation of R values; hence, an adsorbent which strongly adsorbs any and all members of a series of compounds at the very top of the column would be of little value to the chromatographer attempting to determine more than the compound class present on the column.

CHAPTER II

CHEMICALS AND EQUIPMENT

Chemicals

Florisil.--Florisil (a synthetic magnesium silicate, 100 mesh), obtained from the Floridin Company, Tallahassee, Florida, was ground for three hours in a ball mill and used without further treatment. The particle size distribution of the Florisil is listed in Table 1. Adsorbent characteristics of the Florisil, in the manner of LeRosen and his associates (11), are: A = 7000, D^H = 150. A is defined as the acceptor strength of a substance for an electron pair and D^H is defined as the donor strength in terms of an electron pair donated to a hydrogen atom in hydrogen bond formation.

Silicic acid.--Merck Reagent grade silicic acid was ground in a ball mill for three hours and used without further treatment. The particle size distribution of the silicic acid is listed in Table 1.

Aluminum oxide.--Merck Reagent Aluminum Oxide for Chromatographic Purposes was ground in a ball mill to pass a 170 mesh sieve and used without further treatment.

Benzene.--Merck Reagent grade benzene, thiophene free, was used without further treatment.

Hydroxylamine hydrochloride.--Hydroxylamine hydrochloride, obtained from Matheson, Coleman and Bell, was used as obtained.

Sodium hydroxide.--C. P. sodium hydroxide was used as obtained from the J. T. Baker Chemical Company.

Iron wire.--Analytical Reagent grade iron wire was used as obtained from the Mallinckrodt Chemical Works.

Perchloric acid.--"Baker Analyzed" Reagent perchloric acid was used as obtained from the J. T. Baker Chemical Company.

Methanol.--Commercial grade methanol was refluxed with magnesium turnings and then the methanol was distilled from the mixture (12).

Ethyl hexanoate.--Eastman technical grade ethyl hexanoate was washed with dilute aqueous bicarbonate, washed twice with distilled water, and then the entire washing procedure was repeated again. The organic layer was dried over anhydrous sodium sulfate and then distilled. A center cut of the fraction boiling at 165.5° at 741 mm. pressure was taken as pure ethyl hexanoate. The recorded boiling point of ethyl hexanoate or ethyl caproate is 166.6° at 760 mm. (13).

Ethyl oleate.--Some ethyl oleate of unknown purity was obtained from a stockroom bottle. The ester was rinsed with distilled water several times, then with dilute aqueous sodium bicarbonate, and finally with distilled water until the wash water was neutral to litmus. The organic layer was dried over anhydrous magnesium sulfate and then distilled under reduced pressure. A center portion of the fraction boiling at 219° at 18 mm. of mercury was taken as ethyl oleate. The recorded boiling point of ethyl oleate is 220° at 20 mm. pressure (14).

Ethyl α-bromoisobutyrate.--Ethyl α-bromoisobutyrate of unknown purity from Sapon Laboratories was washed with dilute aqueous sodium bicarbonate, washed with two portions of distilled water, and then the washing sequence was repeated. The organic layer was dried over anhydrous

sodium sulfate and then distilled. A center cut of the fraction boiling at 163° at 743 mm. pressure was taken as the pure ester. The recorded boiling point of the ester is 163.6° at 761.9 mm. of mercury (15).

The following esters were obtained as Eastman White Label grade chemicals and used without further purification: benzyl acetate, diethyl maleate, diethyl malonate, diethyl oxalate, di-n-butyl phthalate, ethyl acetate, ethyl α-bromopropionate, ethyl n-butyrate, ethyl cinnamate, ethyl n-decanoate, ethyl formate, ethyl n-heptanoate, ethyl isovalerate, ethyl laurate, ethyl n-nonanoate, ethyl n-octanoate, ethyl palmitate, ethyl propionate, ethyl trichloroacetate, ethyl n-valerate, isobutyl propionate, and triethyl citrate.

The following esters were kindly supplied by Mr. N. H. Horton and used as obtained: α-chloroethyl chloroacetate, β-chloroethyl chloroacetate, β-chloroisopropyl chloroacetate, γ-chloro-n-propyl chloroacetate, isopropyl chloroacetate, n-propyl chloroacetate.

Solutions

Ferric perchlorate.--This solution was prepared by dissolving 0.8 g. of C. P. iron wire in 10 ml. of warm 70 per cent perchloric acid. The solution was cooled and made up to 100 ml. in a volumetric flask with ethanol.

Hydroxylamine hydrochloride.--This solution was prepared by dissolving 12.5 g. of hydroxylamine hydrochloride in 100 ml. of methanol.

Sodium hydroxide.--This solution was prepared by dissolving 12.5 g. of C. P. sodium hydroxide in 100 ml. of methanol by refluxing for one hour.

Ester Streak Reagent A.--This solution was prepared by mixing 5 ml. each of the methanolic solutions of sodium hydroxide and hydroxylamine hydrochloride. The sodium chloride which precipitated was removed by filtration and the solution was used as Ester Streak Reagent A. This solution was stable for approximately four hours.

Ester Streak Reagent B.--This solution was prepared by mixing 5 ml. of the ferric perchlorate solution with one ml. of 70 per cent perchloric acid.

Solutions of the esters.--Small samples of the esters were weighed into glass-stoppered Erlenmeyer flasks. Benzene was added from a burette to the flasks to make 0.01 M solutions.

Equipment

Vacuum pump.--A Welsh Model 1404H pump, obtained from the W. W. Welsh Scientific Company was used in this work. Any good pump capable of producing and maintaining a pressure of five millimeters of mercury in the system will prove satisfactory.

Infrared lamp.--A Rex-Ray Infrared Heat Lamp, X 167, purchased from the Rexall Drug Company, was used in this work as a source of heat.

Chromatographic tubes.--Borosilicate glass chromatographic tubes, No. 1, precision tapered, were obtained from the Scientific Glass Company, Bloomfield, New Jersey. The tubes were tapered 0.00385 mm. outside diameter per millimeter of length. The tubes were 130 mm. long and were fitted with Standard Taper 10/18 outer joints at the bottoms.

Table 1. Particle Size Distribution of Adsorbents

Silicic Acid

Screen Size (U. S.)	Weight of Adsorbent Retained on Screen from a 100 g. Sample
80	0.64 g.
100	17.60 g.
140	56.87 g.
170	14.44 g.
200	0.42 g.
325	2.12 g.
> 325	7.04 g.

Florisil

Screen Size (U. S.)	Weight of Adsorbent Retained on Screen from a 100 g. Sample
80	14.23 g.
100	36.31 g.
140	30.91 g.
170	7.80 g.
200	2.34 g.
325	5.38 g.
> 325	1.89 g.

CHAPTER III

PROCEDURE

The apparatus for chromatography was assembled in the following manner. The vacuum pump was connected through a three-way stopcock to a surge tank (the three-way stopcock allowed the pump to be vented to the atmosphere when not in use). The surge tank was connected in turn to a series of T-tubes and ultimately to a manometer. From each T-tube, pressure tubing led through a three-way stopcock to a suction flask supporting the chromatographic apparatus proper.

The lower section of the chromatographic apparatus was set in a one-hole rubber stopper in the mouth of the suction flask. This lower section was simply a tube, topped by a Standard Taper 10/18 inner joint and a perforated glass disc insert. The chromatographic tube proper, or upper section of the apparatus, then fitted onto this lower portion by means of a Standard Taper 10/18 outer joint. The glass disc insert in the lower section provided a base or support for a cotton wad which in turn supported the adsorbent.

It was desirable to have all of the columns of adsorbent of approximately equal height. This was achieved by practice in filling the tubes with the adsorbent such that the final column length after packing was 75 ± 5 mm. The adsorbents were stored in 250 ml. wide-mouth bottles provided with a one-hold rubber stopper. A glass tube, 10 mm. inside diameter, 10 cm. long, runs through the stopper to a

piece of thin-walled rubber tubing five cm. long, closed by a pinch clamp.

The following procedure was employed in this laboratory for filling the column with adsorbent. A small wad of surgical cotton was tamped down into the bottom of the chromatographic tube. Adsorbent was delivered from the storage bottle into the tube by removing the pinch clamp from the rubber tubing on the bottle, affixing the rubber tubing to the top of the chromatographic tube, and shaking the bottle until the desired amount of adsorbent had been delivered into the tube. Uniform packing was accomplished by tapping the tube vigorously with a dowel rod while applying the full vacuum of the available pump. It is not desirable to add more adosrbent once the vacuum packing of the adsorbent has begun. Finally, the top of the column was leveled with the dowel rod without exerting pressure on the adsorbent.

With the vacuum still applied, a 0.5 ml. sample of the solution to be chromatographed was pipetted onto the top of the packed column and followed immediately by a small portion of the developing solvent, benzene. The ester was worked onto the column with several small volume increments of benzene. Finally the tube was filled to the top with benzene and development proceeded until the developer reached the bottom of the column. The volume of benzene required to develop a column 75 ± 5 mm. long is 2.5 ± 0.2 ml.

The vacuum was disconnected by means of a three-way stopcock which simultaneously vented the suction flask holding the chromatographic tube to the atmosphere. The tube was then lifted from the

apparatus and tapped on a folded towel until the column of adsorbent began to slide out. The column was then completely extruded with a dowel rod onto a smooth surface such as a burette stand base or onto a porcelain pipette rest. The latter was especially useful for the heating process which followed.

The column was streaked first with Ester Streak Reagent A and then placed under an infrared lamp for two minutes. The lamp was positioned at a height of 33 mm. above the column and a temperature of 135° was measured at the column surface. The column was then over-streaked with Ester Streak Reagent B. Both streakings were accomplished with an eye dropper which had been drawn out to a fine tip. The use of the fine tip enabled one to streak a very narrow area and use a minimum of streak reagent. This procedure gave rise to more sharply defined zones. Since the column was heated and streaked twice, gentle handling was necessary.

When the purple zone identifying an ester appeared, measurements were made to determine the R value. A ruler graduated in millimeters was laid beside the column, and the column length and the distances from the top of the column to both edges of the zone were recorded. To determine the R value for the ester, the distance from the top of the column to the bottom of the purple zone was divided by the length of the column. Thus we had the ratio of the distance traveled by the ester to the distance traveled by the developing solvent.

At least two columns were used for each ester and the R values were averaged, with the deviation from the average of a single run being

no more than 0.02. The infrared lamp was firmly clamped in position throughout the work. The chromatographic columns were placed on a pipette rest, streaked with Ester Streak Reagent A, and then the pipette rest was placed on the base of the stand holding the lamp.

Studies conducted in this laboratory have indicated that there was no measurable difference in R values when a given compound was chromatographed using either straight or precision tapered chromatographic tubes. It is quite easy to remove the developed column from the precision tapered tube after gently tapping the top end of the tube on a folded towel. These studies were conducted with a 0.01 M solution of phenol in benzene, using benzene as developer, and silicic acid as adsorbent. The streak reagent used to detect phenol was alkaline permanganate (5), which gave brown zones for the phenol against a purple background.

CHAPTER IV

DISCUSSION OF RESULTS

The R values obtained for the esters used in this study are listed in Tables 2, 3, and 4. Table 2 contains the homologous series of ethyl esters chromatographed on Florisil. Table 3 lists the R values of some branched chain and substituted esters which were chromatographed. Table 4 contains a short series of acetates and other assorted esters which were used to test the applicability of the detecting reagents.

The R values for the homologous series of ethyl esters (see Table 2) show a general increase as the acid portion of the ester increases in molecular weight. Exceptions to this tendency are ethyl formate and ethyl hexanoate. Ethyl formate has a higher R value than would be expected for the lowest member of this series. Ethyl hexanoate has a slightly lower R value than one might expect, but this R value is not nearly so far out of line as is the R value for ethyl formate.

It might be noted at this point, in connection with the low R value for ethyl hexanoate, that Smith and LeRosen (16) found decreases in R value at two places in the chromatographic behavior of a homologous series of straight-chain methyl ketones. When R values were plotted against the number of carbon atoms in the ketones, distinct dips in the curve were noted at C_8 and C_{14}. Both R_l nad R_t values showed this decrease in R value at C_8 and C_{14} (the subscripts $\underline{l}$ and $\underline{t}$ refer to leading and trailing edges of the zones, respectively).

Table 2. R Values of a Homologous Series of Ethyl Esters

Ester	R
Ethyl formate	0.23
Ethyl acetate	0.16
Ethyl propionate	0.18
Ethyl n-butyrate	0.20
Ethyl n-valerate	0.21
Ethyl n-hexanoate	0.19
Ethyl n-heptanoate	0.23
Ethyl n-octanoate	0.30
Ethyl n-nonanoate	0.30
Ethyl n-decanoate	0.30
Ethyl laurate	0.31
Ethyl palmitate	0.35

Table 3. R Values of Some Branched Chain and Substituted Esters

Ester	R
Isobutyl propionate	0.27
Methyl chloroacetate	0.34
α-Chloroethyl chloroacetate	NR
β-Chloroethyl chloroacetate	0.34
n-Propyl chloroacetate	0.46
γ-Chloro-n-propyl chloroacetate	0.35
Isopropyl chloroacetate	0.35
β-Chloroisopropyl chloroacetate	0.42
Ethyl α-bromopropionate	0.46
Ethyl α-bromoisobutyrate	NR
Ethyl isovalerate	NR
Ethyl trichloroacetate	0.85

No reaction was observed on the column.

Table 4. R Values of Some Acetates and Other Available Esters

Ester	R
Ethyl acetate	0.16
n-Propyl acetate	0.22
n-Butyl acetate	0.26
n-Amyl acetate	0.31
Benzyl acetate	0.25
Ethyl oleate	0.28
Ethyl cinnamate	0.17
Diethyl oxalate	0.08
Diethyl malonate	0.08
Triethyl citrate	0.08
Diethyl maleate	0.12
Di-n-butyl phthalate	0.11

The carbonyl oxygen atom is considered to be the principal adsorbing atom for the esters. Considering the possible mesomeric effects, one would deduce that there would be little difference in polarity of the oxygen among the various homologous ethyl esters, since the mesomeric effect should be of the same order of size for each of the esters. The increasing molecular weight of the side chain would tend to make R values higher as one proceeds to the higher members of the series of homologous ethyl esters.

The R values listed in Table 3 seem to indicate that substituents and branched side chains do affect the R values of the esters, although the exact nature of these effects is not obvious. There are three esters in this series which did not give color reactions under the conditions of the test. These three esters did give purple colors when the pure esters were treated on a spot plate as outlined in the procedure for testing for esters. Hence it seems probable that the lower detection limit for these particular esters was not exceeded in the 0.01 M solutions used in this study.

No general conclusion has been drawn concerning the steric factors for the branched chain esters. The chlorine-substituted esters indicate no noticeable trend other than that these substituted esters have larger R values than unsubstituted esters with the same number of carbon atoms. The chlorine atom could be involved in steric hindrance of the carbonyl oxygen, the chlorine atom itself could be adsorbed, or by virtue of its electronegativity it may exert inductive influences on the adsorbing atom which would decrease the affinity of the molecule

for adsorption. The size of the chlorine atom does not suggest any particular potential for it as a blocking atom, nor is there evidence that the chlorine atom has more than very slight affinity for adsorption columns. The electronegativity of the chlorine atom would reduce the electron density about the carbonyl oxygen, thus lowering its adsorption affinity. This is reflected in the R values for n-propyl chloroacetate and ethyl trichloroacetate. From Table 4, the R value for n-propyl acetate is 0.22, while from Table 3 the R value for n-propyl chloroacetate is 0.46. From Table 2, the R value for ethyl acetate is 0.16, while from Table 3, the R value for ethyl trichloroacetate is 0.85.

The short series of acetates listed in Table 4 shows a very regular increase in R values as the molecular weight of the alcohol portion of the ester increases. The increase in R value per additional carbon atom is higher for the acetates than for the homologous series of ethyl esters.

The esters of di- and tribasic acids (see Table 4) were all strongly adsorbed at the top of the column and had R_t values of zero. This behavior lends support to the contention that the carbonyl oxygen atom is the principal adsorbing atom, since monoesters of similar molecular weight move much further down the column.

Unsaturation in aliphatic portions of the ester seems to lead to lower R values, as with ethyl oleate and ethyl cinnamate. Since the process of adsorption is considered as taking place by bonding to electron acceptor by a donor, increased unsaturation should lead to still lower R values.

In a mixture of organic substances one would have interference from acid halides and acid amides since these substances give colored reaction products under the conditions of the test used for esters. The lower members of the acid halides and acid amides are quite water soluble and could be removed by treatment of the organic mixture with water. A sodium fusion test would indicate the presence of elements other than carbon, hydrogen, and oxygen so as to alert the experimentor to the nature of the compounds present. Aldehydes can form hydroxamic acids when treated with the sodium salt of nitrohydroxylamine. Ketones do not form hydroxamic acids.

Primary aliphatic amines give hydroxamic acids when treated with monopersulfuric acid (Caro's acid). Primary aliphatic nitro compounds are believed to form hydroxamic acids as intermediates when boiled with sulfuric acid to form carboxylic acids. However, the conditions for these reactions are not met in the ester test.

The work undertaken in this study was designed primarily to find a system of reagents which would give an easily discernable color reaction with esters in general. Detection of esters as the ferric hydroxamates can be easily performed when Florisil is used as the adsorbent. Silicic acid and alumina both proved unsatisfactory when the reagents were unable to bring out the position of the ester on the columns. Possibly, the sodium hydroxide present in the reagent to saponify the ester was lost by reaction with the adsorbent. Finely ground alumina was very slow in the developing process, and the columns were not firm when extruded. Florisil, in addition to bringing out the ester zone,

was easy to handle, held the column shape well when extruded, and had a short developing time.

A paper embodying the techniques for detecting esters on Florisil has been published by Dr. Jack K. Carlton and the author (17).

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PART II

INVESTIGATION OF THE STERIC EFFECT OF AIKYL GROUPS ON THE ADSORPTION OF ANILINES

CHAPTER I

INTRODUCTION

Carlton and Bradbury (1) have indicated that the steric hindrance of the alkyl substituent is the most important influence of that group on the chromatographic adsorption of ortho-alkyl substituted phenols. Earlier investigators (2, 3, 4) have mentioned steric hindrance, along with internal hydrogen bonding, inductive effects, and resonance effects as contributing to the chromatographic behavior of substituted aromatic compounds. LeRosen, Carlton, and Moseley (5) chromatographed a series of ortho- and para-disubstituted benzene compounds, but the influence exerted by the second substituent was such as to enhance or impair the normal adsorption affinity of the first functional group in such a manner as to preclude qualitative evaluation of the steric factors involved.

The investigation of substituted anilines was undertaken in an attempt to elucidate the steric effect of alkyl groups on the adsorption properties of the amino group attached to a benzene nucleus. Alkyl groups were chosen as the second substituent for this work for the reason that these substituents would partially block adsorption of the functional group by spatial configuration and at the same time offer minimum inductive and resonance effects. Another purpose of this investigation was to correlate the sizes of alkyl and non-alkyl groups, and from this information and R values, deduce the steric interference

of the non-alkyl group, as well as a relative value of other effects such as the inductive effect, resonance, and possibly the steric inhibition of resonance.

If the substituent is in the para position, an electron donating effect would increase the electron density about the nitrogen and thus increase adsorption affinity (decrease the R value), assuming adsorption through the electron pair on nitrogen. Conversely, an electron withdrawing substituent in the para position would decrease the electron density around the nitrogen atom and lead to a higher R value. There should be no steric hindrance from the para substituent.

When the substituent is in the ortho position, one has the problem of separating the steric effect from any inductive effect. By a comparison of sizes and R values of several groups, one should be able to indicate which of the effects is of major importance and then assign at least qualitative values to the various effects.

One of the essential assumptions of chromatography is that there is a continual dynamic equilibrium involving the adsorptive, the adsorbent, and the developing solvent. LeRosen and coworkers (5) have considered the effect of the solvent on adsorption behavior on the basis of polarity of the adsorptive and of the solvent. They have noted that one would expect incompatibility between a highly polar adsorptive and a non-polar solvent. Thus a highly polar adsorptive would spend more time on the adsorbent and this, of course, would cause a low R value.

Yet another factor in adsorption considered by LeRosen and his coworkers (5) was the possibility that the spatial arrangement of donor or acceptor sites on the ring may be such as to allow a close fit with adsorption sites on the adsorbent, while in others the spatial arrangement may allow adsorption of only one group at a time. This factor could account for abnormal adsorption behavior of various disubstituted compounds on a particular adsorbent, and it would be significant in explaining variations in R value of a given disubstituted compound on different adsorbents.

Unpublished results of Dr. Jack K. Carlton have indicated that silicic acid containing approximately 12.5 per cent water would be suitable for separation of various substituted anilines. Experimentation with the water content of Florisil in conjunction with the study of substituted anilines has indicated that a water content near 2.5 per cent on Florisil gives an adsorbent which closely approaches the silicic acid used in adsorption of the anilines.

CHAPTER II

CHEMICALS AND EQUIPMENT

Chemicals

The following chemicals were obtained as Eastman White Label grade and were used as obtained from the supplier: N,N-dimethylaniline (monomethylaniline free), N-methylaniline (aniline and dimethylaniline free), 2,6-dimethylaniline, p-toluidine, o-nitroaniline, m-nitroaniline, p-nitroaniline, o-phenylenediamine, p-phenylenediamine, o-chloroaniline, m-chloroaniline, p-chloroaniline.

Aniline.--Eastman White Label grade aniline from an open stock bottle was distilled from zinc dust at reduced pressure. The middle portion of the clear distillate was then used to make up the aniline solution.

2,4,6-Tri-t-butylaniline.--2,4,6-Tri-t-butylaniline was prepared by the method of Bartlett, Roha, and Stiles (6) from a sample of 1,3,5-tri-t-butylbenzene kindly supplied by the Standard Oil Company of Indiana. The melting point of the amine prepared in this laboratory was 144-145°; the literature value was 144.5-145.5° (6).

o-t-Butylaniline.--o-t-Butylnitrobenzene was prepared by the method of Craig (7). The boiling point of this compound was 139-140° at 21 mm. pressure; the literature value was 140-141° at 21 mm. pressure (6). The refractive index of o-t-butylnitrobenzene was 1.5169 at 26°. The nitro compound was then reduced to o-t-butylaniline by the method of Bartlett, Roha, and Stiles (6). The o-t-butylaniline boiled at 120-121°

at 21 mm. pressure; the boiling point listed in the literature was 121-122° at 21 mm. pressure (6). An acetyl derivative melted at 160-161°; the literature melting point was 158-161° (6).

p-t-Butylaniline.--p-t-Butylnitrobenzene was prepared by the method of Craig (7). The boiling point of this compound was 149-150° at 21 mm. pressure; the literature value was 150° at 21 mm. pressure (6). The refractive index was 1.5388 at 26°. The nitro compound was then reduced to p-t-butylaniline by the method of Bartlett, Roha, and Stiles (6). The p-t-butylaniline boiled at 125-126° at 21 mm. pressure; the literature value was 125-126° at 21 mm. pressure (6). An acetyl derivative melted at 169°; the literature value was 169-170° (6).

o-Isopropylaniline.--o-Nitroisopropylbenzene (o-nitrocumene) was prepared by the method of Haworth and Barker (8). The nitro compound boiled at 116-117° at 17 mm. pressure; the literature value was 115-120° at 20 mm. pressure (8). The refractive index was 1.5222 at 26°. The nitro compound was then reduced to o-isopropylaniline by the method of Bartlett, Roha, and Stiles (6). The o-isopropylaniline boiled at 96-97° at 19 mm. pressure and at 215-216° at 741 mm. pressure, discoloring rapidly at the latter pressure. The literature value of the boiling point of the aniline was 213.5-214.5° at 732 mm. pressure (9). An acetyl derivative melted at 71°; the literature melting point was 72° (9).

p-Isopropylaniline.--p-Nitroisopropylbenzene was prepared by the method of Haworth and Barker (8). The boiling point of this compound was 130-132° at 16 mm. pressure; the literature value was 128-132° at 15 mm.

pressure (8). The refractive index was 1.5348 at 26°. The nitro compound was reduced to p-isopropylaniline by the method of Bartlett, Roha, and Stiles (6). The amine boiled at 103-104° at 19 mm. pressure; the literature value was 103-105° at 20 mm. pressure (8). An acetyl derivative melted at 102°; the literature value for the melting point was 102-102.5° (10).

2,6-Diethylaniline.--A sample of Eastman Practical grade 2,6-diethylaniline was taken up in ether, and dry HCl gas was passed into the solution. The solid which formed was washed several times with ether, left under ether overnight, and finally washed twice more with ether. The amine was liberated in water with sodium hydroxide and then taken up with ether. The ether was evaporated and the amine was then distilled over zinc dust. A center portion of the clear distillate boiling at 126-127° at 17 mm. pressure was taken as 2,6-diethylaniline. No literature data on this compound were found by this author and a private communication from the Eastman Organic Chemicals Department advises that they can likewise find no data in the literature. An acetyl derivative prepared in this laboratory from the purified compound melted at 139.5-140.5°. The refractive index of the purified amine was 1.5426 at 26°.

o-Ethylaniline.--A sample of Eastman Practical grade o-ethylaniline was taken up in ether and dry HCl gas was passed into the solution. The precipitate was washed with ether, stored under ether overnight, and washed twice more with ether. The amine was liberated in water with sodium hydroxide and taken up in ether. The ether was evaporated

and then the amine was distilled over zinc dust. A portion of the clear distillate boiling at 214-215° at 742 mm. pressure was taken as o-ethylaniline; the boiling point listed in the literature is 216° (10). The acetyl derivative of the amine melted at 111°; the literature value was 111° (10).

o-Toluidine.--A sample of o-toluidine of unknown purity was taken from a stock bottle and dissolved in ether. Sufficient oxalic acid was added to precipitate half of the amine, which would preferentially precipitate p-toluidine if any were present due to the higher solubility of the o-toluidine. The first precipitate was filtered and discarded, and more oxalic acid was added to the filtrate. This second precipitate was then washed with ether, stored under ether overnight, and washed twice more with ether. The amine was then liberated in water with sodium hydroxide and taken up in ether. The ether was evaporated, and the amine was distilled over zinc dust. A portion of the clear distillate boiling at 198-199° at 743 mm. pressure was taken as o-toluidine; the literature value for the boiling point of o-toluidine was 200° (10). The acetyl derivative of the amine melted at 111-112°; the literature value was 112° (10).

Silicic acid.--Merck Reagent grade silicic acid was ground in a ball mill for three hours and then dried in an oven at 145° for 30 hours. The silicic acid was then placed in a horizontally rotating cylinder and an amount of water added such that the silicic acid would contain 12.5 per cent water by weight. The cylinder was then rotated for three hours to insure uniform mixing. The silicic acid was sampled for

water determination at the end of the mixing period, and the samples were dried at 145° for at least 18 hours. Loss in weight over the drying period determined the water content of the samples. The silicic acid used in this study contained 12.75 per cent by weight of water.

Florisil.--Florisil from the Floridin Company, Tallahassee, Florida, was ground for three hours in a ball mill and then dried in an oven at 145° for 30 hours. The Florisil was then placed in the horizontally rotating cylinder and treated by the same procedure as used for the silicic acid above. The Florisil thus obtained had a water content of 11.38 per cent water by weight. Water determinations were made on milled but undried Florisil. One such batch of Florisil contained 2.35 per cent water and another batch contained 3.22 per cent water.

Benzene.--Merck Reagent grade benzene, thiophene free, was used as obtained.

Solutions

Ceric nitrate.--Ceric nitrate was prepared according to the directions of LeRosen, Moravek, and Carlton (11).

p-Nitrobenzenediazonium tetrafluoroborate.--This compound was prepared according to the instructions of Cheronis and Entrikin (12). Fresh one per cent solutions were made up in water daily.

Solutions of the anilines.--Small samples of the anilines were weighed into glass-stoppered Erlenmeyer flasks. Benzene was added from a burette to the flasks to make 0.01 M solutions.

Equipment

The equipment used in this study was the same as the equipment used in Part I of this thesis.

CHAPTER III

PROCEDURE

The procedures for packing the columns, adding developing solvent, extruding the columns, and streaking the columns were the same as those in Part I of this thesis. Sample size of the benzene solutions of the anilines was 0.2 ml. since the 0.5 ml. sample normally used gave too wide a zone. As long as the zone is detectable by the use of streak reagents, the volume of solution used will not affect the relative R values if 0.01 M solutions are used in all cases.

At least duplicate columns were used for each of the anilines and the R values were averaged with the deviation from the average of a single run being no more than 0.02. Two streak reagents were used on each column, ceric nitrate and p-nitrobenzenediazonium tetrafluoroborate. By careful application of the streak reagents, each column was streaked from four to six times. Chloranil in dioxane was used as a streak reagent at the beginning of the investigation, but these preliminary trials gave only faint gray zones which were difficult to measure with accuracy.

A sample of each aniline was used with benzene developer for each of the adsorbents. Silicic acid with 12.75 per cent water, Florisil with 11.38 per cent water, and Florisil with 3.22 per cent water were all used for almost the entire series of anilines. Florisil

with 2.35 per cent water was used for a few of the alkyl-substituted anilines to compare Florisil of this water content to Florisil bearing 3.22 per cent water. The final column length after packing was 75 ± 5 mm.

All melting points were taken with the aid of a microscope and a Kofler Hot Stage melting point apparatus. The melting points listed are uncorrected.

CHAPTER IV

DISCUSSION OF RESULTS

The R values obtained from the chromatography of the anilines are listed in Tables 1 through 4. The R values listed in Tables 1 and 3 are those of the ortho- and para-substituted anilines, while Tables 2 and 4 list the R values of some other anilines of interest which will contribute to our understanding of the chromatographic behavior of the anilines.

Examination of the R values in the tables will reveal that R values and base strengths expressed in pK units cannot be correlated directly without consideration of other factors. It can be seen from Tables 1 and 2 that aniline, N-methylaniline, and N,N-dimethylaniline have very similar basic strengths as determined by their pK values. Yet we see from Table 2 that the R values of N-methyl aniline and N,N-dimethylaniline are nearly the same, but that these R values are twice that of aniline itself.

While one cannot determine the basic strength of a substituted aniline and immediately predict its behavior on an adsorbent, it would be incorrect to conclude that there is no correlation between the R value and the availability of the electron pair on the nitrogen atom. While some of the factors which contribute to the behavior of compounds on adsorbents are not well understood, one must attempt to obtain a better understanding if our present information is to be utilized completely.

Consider the ortho-substituted anilines whose R values on silicic acid are listed in Table 1. As the size of the alkyl group in the ortho position increases, the R value increases, indicating that the larger alkyl groups are somehow interfering with the process of adsorption of the amino group. In 1930, Bennett and Moses (13) assumed an unusual electron withdrawing effect for the methyl group to explain the decreased basicity of o-toluidine compared to aniline. Brown and Cahn (14) have observed that steric strain would appear to offer a much simpler interpretation, since the formation of the anilinium-type ion would necessitate the formation of a tetrahedral nitrogen atom. In the ortho-alkyl aniline series, steric hindrance seems to offer a reasonable interpretation of the data. Within this particular series there appears to be some correlation of R value with pK value. For example, aniline has an R value of 0.25 and a pK_a value of 4.58 (15); o-toluidine has an R value of 0.30 and a pK_a value of 4.39 (15); o-t-butylaniline has an R value of 0.55 and a pK_a value of 3.78 (16). The pK_a values just cited were obtained in water at 25°.

Examination of molecular models indicates that the spatial configuration of the ortho-alkyl group is such in the larger groups that the nitrogen is effectively blocked toward adsorption on one side. This information agrees with the conclusion drawn by Carlton and Bradbury (1) as to the effect of the ortho-alkyl group on the adsorption of phenols, although the same alkyl group does not affect the extent of adsorption to the same degree. It has also been determined that the basic strength of 2-alkylpyridines decreased as the size of the alkyl group increased (17).

Table 1. R Values and Some pK_a Values of Ortho- and Para-Substituted Anilines on Silicic Acid Adsorbent

Compound	R	pK_a
Aniline	0.25	4.58[1], 4.14[2]
o-Toluidine	0.30	4.39[1]
p-Toluidine	0.20	5.12[1]
o-Ethylaniline	0.34	
p-Ethylaniline	0.21	
o-Isopropylaniline	0.37	
p-Isopropylaniline	0.25	
o-t-Butylaniline	0.55	3.78[1], 3.39[2]
p-t-Butylaniline	0.25	4.00[2]
o-Nitroaniline	0.50	
p-Nitroaniline	0.20	2.00[1]
o-Phenylenediamine	0.04	
p-Phenylenediamine	0.08	
o-Chloroaniline	0.72	2.13[3]
p-Chloroaniline	0.42	3.46[3]

[1] In water at 25°.

[2] In 90 per cent methanol.

[3] In 50 per cent aqueous ethanol.

Table 2. R Values and Some pK_a Values of Other Anilines on Silicic Acid Adsorbent

Compound	R	pK_a
Aniline	0.25	4.58[1]
m-Nitroaniline	0.27	
m-Chloroaniline	0.50	2.93[2]
2,6-Dimethylaniline	0.35	3.42[2]
2,6-Diethylaniline	0.51	
2,4,6-Tri-t-butylaniline	1.00	2.00[3]
N-Methylaniline	0.50	4.40[1]
N,N-Dimethylaniline	0.56	4.26[2]

[1] In water at 25°.

[2] In 50 per cent aqueous ethanol.

[3] In 90 per cent methanol.

From Table 1 it may be seen that the positioning of the alkyl group in the para position has quite a different effect than when the group is in the ortho position. Perhaps this effect could have been predicted since it has been determined that para-toluidine is a stronger base than aniline by 0.54 pK units in water (14), while para-t-butyl-aniline is a weaker base than aniline by only 0.14 pK units in 90 per cent methanol (6). The R values listed for the para-alkyl aniline series are the same within the limits of experimental error, however. From electronic theory one would expect p-t-butylaniline to be a slightly stronger base than aniline. It may be that the increased molecular weight of p-t-butylaniline causes its R value to increase slightly and thus be the same as the R value of aniline itself.

For the following discussion, size comparison will refer to the volume generated by the revolution of the group attached to the aromatic nucleus around the axis of the bond connecting the first member of the group to the aromatic nucleus. The nitro group falls between methyl and ethyl groups in size, yet the R value of o-nitro-aniline is almost the same as that of o-t-butylaniline. This difference seems to indicate that the nitro group has a strong electron withdrawing force which is quite active in the ortho position. p-Nitro-aniline, which is almost three pK units less basic than aniline, has an R value smaller than aniline. While we still must consider the nitro group to be an electron withdrawing group, it seems logical to conclude that in this particular case, the spatial arrangement of the groups on the ring is such that both groups can be adsorbed on sites on the

adsorbent. While the nitro group itself shows little tendency to be adsorbed, as derived from the behavior of nitrobenzene, it may be more strongly adsorbed when its electron density is increased as in the para position of p-nitroaniline. Although the nitro group may increase its electron density at the expense of the amino nitrogen atom, this enrichment should not completely preclude the ability of the amino nitrogen atom to participate in adsorption. This decrease in electron density around the amino nitrogen would increase the probability of hydrogen bonding between the amino hydrogens and donor sites on the adsorbent.

Yet another factor worthy of consideration in the case of o-nitroaniline, is internal hydrogen bonding, which would increase the R value of the compound since an amino hydrogen, thus participating in internal effects, will not be effective in adsorption; likewise, one of the nitro group oxygen atoms will be involved in the bonding process. Taking the R value of o-ethylaniline as being the maximum value for steric effects only of the nitro group, the combined other effects would have a value of 0.16 $\pm$ 0.04 R value units. This will be designated as +0.16 since the observed R value is higher than the one associated only with steric factors.

From Table 1, the R value of o-chloroaniline is 0.72 and the R value of p-chloroaniline is 0.42. In size, the chlorine atom is smaller than the methyl group, and if the only effect of the chlorine atom were a steric effect, then the R value of o-chloroaniline would be between 0.25 and 0.30. That o- and p-chloroaniline are weaker bases

than aniline is readily understood since the chlorine atom has an electron withdrawing effect. Taking the R value of o-toluidine as the maximum value which o-chloroaniline would have if the chlorine atom did nothing more than spatially interfere with the adsorption of the amino group, we arrive at a value for these effects of 0.42 ± 0.04.

The magnitude of the various effects other than steric hindrance for the ortho-chloro group is large compared to the ortho-nitro group, even when internal hydrogen bonding is postulated for the nitro group. The most plausible explanation for this large difference would seem to lie in the relative polarities of the two groups. LeRosen and his co-workers (5) have observed that one would expect incompatibility between a highly polar adsorptive and a non-polar solvent. A highly polar compound would be only slightly soluble in a non-polar solvent, and, in the competitive equilibria involved in the adsorption process, the adsorptive would spend more time on the adsorbent than in the non-polar solvent. This would lead to a low R value for the polar compound. One basis for discussion of relative polarities would involve dipole moments. The dipole moment of nitrobenzene in benzene is 3.97 D. while the dipole moment of chlorobenzene in benzene is 1.56 D. (18). Since the nitro group has an electron withdrawing effect and the amino group has an electron donating effect, the two groups can be in conjugation with the ring as well as with one another, as pointed out by Ingold (19). o-Nitroaniline is more soluble in benzene than p-nitroaniline, and thus the para compound would spend more time on the adsorbent than in the non-polar solvent benzene, resulting in a smaller R value.

p-Chloroaniline is less soluble in benzene than o-chloroaniline and hence would spend more time on the adsorbent than in solution to give a lower R value, if one considered only the incompatibility between polar adsorptive and non-polar solvent. Although the steric hindrance and electron withdrawing effect are less for the chlorine than for the nitro group, o-chloroaniline has a higher R value than o-nitroaniline. Hence o-chloroaniline must be more soluble in benzene and less polar than o-nitroaniline. The consideration of solubility as the major influence will explain the high R value for the chloro compound.

The ortho- and para-phenylenediamines were strongly adsorbed at the tops of the adsorbent columns. Apparently the ortho compound is more strongly adsorbed on silicic acid, although the R values are the same within the experimental error. Strong adsorption is not surprising with two strongly adsorbing groups on the benzene nucleus. The zones on the columns were narrow and quite distinct at their edges.

When identical groups are substituted in the 2- and 6-positions on aniline, one would expect the steric effect of the two groups to be more than twice as great as the steric effect of one of these groups in the ortho position. This appears to be the case for the 2,6-disubstituted anilines when the substituents are alkyl groups. Aniline has an R value of 0.25, o-toluidine has an R value of 0.30, and 2,6-dimethylaniline has an R value of 0.35. In this particular case, the steric effect of the second methyl group appears to be the same as the effect of the first group. o-Ethylaniline had an R value of 0.34 and 2,6-diethylaniline had an R value of 0.51. It has been shown that a

t-butyl group in the para position has little or no effect on the R value of the monosubstituted anilines, and hence 2,4,6-tri-t-butylaniline should be adsorbed in very nearly the same manner as 2,6-di-t-butylaniline, which was not available for use in this study. The R value of o-t-butylaniline was 0.55 and the R value of 2,4,6-tri-t-butylaniline was 1.00. For the diethyl and tri-t-butyl cases, the second alkyl group does indeed seem to operate with greater efficiency at blocking adsorption of the amino group than does the first group.

Carlton and Bradbury (1) have reported similar results for 2,6-disubstituted phenols. Stillson, Sawyer, and Hunt (20) have prepared a series of hindered phenols which demonstrate a very marked resistance to reaction. Stillson and his coworkers reported that 2,4,6-tri-t-butylphenol formed a benzoate derivative only from liquid ammonia solutions. These workers have also reported that 2,6-di-t-amyl-4-methylphenol, 2,6-di-t-amyl-4-t-butylphenol, and 2,4,6-tri-t-amylphenol did not form benzoates under any conditions tried. Bartlett, Roha, and Stiles (6) have reported that 2,4,6-tri-t-butylaniline did not form any derivatives. Thus R values of 1.00 were definitely expected for these highly hindered compounds.

R values of m-chloroaniline and m-nitroaniline on silicic acid are listed in Table 2. In each case the R value of the meta compound is between the R values of the ortho and para compounds, but nearer the para than the ortho compound. The arguments previously advanced concerning the polarity of the compounds and the incompatibility of a polar compound with a non-polar solvent seem applicable in this case.

It should also be recalled that the resonance effect operates best from the para position.

The chromatographic behavior of the N-substituted anilines was regarded as being highly informative in determining the nature of the adsorption of the amino group. There are two possible explanations for the adsorption of the amino group, both revolving around the availability of the electron pair on the nitrogen atom. There may be adsorption due to the acceptance by a site on the adsorbent of the pair of electrons on the nitrogen atom, or there may be adsorption due to hydrogen bonding between the hydrogen atoms on the amino nitrogen and an electron donor site on the adsorbent. The availability of the electron pair on nitrogen will determine the extent to which the amino hydrogens can form hydrogen bonds with donor sites on the adsorbent since the availability of this electron pair will determine the strength with which the hydrogen atoms are bound to the nitrogen atom.

The R values of N-methylaniline and N,N-dimethylaniline on silicic acid are 0.50 and 0.56, respectively (see Table 2). These data indicate that both factors mentioned immediately above are involved in the adsorption, and also indicate that the types of adsorption are affected to almost the same degree. If the amino hydrogens were the primary sources of adsorption, the R value of N,N-dimethylaniline should be much greater than the R value of N-methylaniline, and would probably approach 1.00. If the electron pair on the amino nitrogen were the principal source of adsorption one

would expect the steric hindrance of two methyl groups to be greater than the steric hindrance of one methyl group. The steric effect of the methyl groups would not be large since the pK's of N,N-dimethylaniline and aniline are almost the same (14). Hence no valid conclusion can be drawn concerning the exact mechanism of adsorption of the anilines.

The cases just considered have involved R values obtained using silicic acid adsorbent with 12.75 per cent water by weight. The same trend of R values is observed on Florisil, as may be noted in Tables 3 and 4. The differences in R values among the series discussed are not the same, but this is not unusual when adsorbents are changed. The R values obtained with Florisil, containing 11.38 per cent water, are, with three exceptions, greater than 0.65. These values are not as reproducible as lower R values. The high R values also indicate that the Florisil containing 11.38 per cent water is less acidic than the Florisil containing 3.22 per cent water. This latter adsorbent gives R values which are more in agreement with the values obtained using the silicic acid. One batch of Florisil containing 2.35 per cent water was tested for adsorption properties with some of the alkyl-substituted anilines, and the R values obtained with this adsorbent were in good agreement with the R values obtained with the silicic acid.

Several points of interest are to be noted in Tables 3 and 4. The Florisil with 11.38 per cent water content seems to give the best separation of the ortho- and para-phenylenediamines of all of the adsorbents used. It is also noteworthy that the order of adsorption of these isomers is reversed between Florisil with the greater water

Table 3. R Values of Some ortho- and para-Substituted Anilines on Florisil Adsorbent

Compound	Florisil (3.22% H_2O) R	Florisil (11.38% H_2O) R
Aniline	0.32	0.72
o-Toluidine	0.35	0.75
p-Toluidine	0.25	0.67
o-Ethylaniline	0.38	0.82
p-Ethylaniline	0.25	0.65
o-Isopropylaniline	0.44	0.82
p-Isopropylaniline	0.27	0.68
o-t-Butylaniline	0.55	0.91
p-t-Butylaniline	0.28	0.67
o-Nitroaniline	0.33	0.70
p-Nitroaniline	0.13	0.37
o-Phenylenediamine	0.04	0.14
p-Phenylenediamine	0.03	0.07
o-Chloroaniline	0.71	0.96
p-Chloroaniline	0.43	0.81

Table 4. R Values of Some Other Anilines on Florisil Adsorbent

Compound	Florisil (3.22% H_2O) R	Florisil (11.38% H_2O) R
Aniline	0.32	0.72
m-Nitroaniline	0.27	--
m-Chloroaniline	0.54	--
2,6-Dimethylaniline	0.40	0.83
2,6-Diethylaniline	0.52	0.93
2,4,6-Tri-t-butylaniline	0.92	1.00
N-Methylaniline	0.57	0.96
N,N-Dimethylaniline	0.57	0.97

content and the silicic acid. While the differences are not large enough to be conclusive, this reversal of adsorption order may very well illustrate that the distance between adsorption sites on the adsorbent and adsorptive may be in such agreement as to have two adsorbing centers on one molecule active on one adsorbent but not on another adsorbent.

Another point of interest in studying the R values obtained on Florisil is that the R values of the N-substituted anilines are the same. While the R values obtained on silicic acid were very close, the R values on Florisil are the same within the experimental error. This seemingly confirms the contention advanced earlier that while the electron pair on the nitrogen or the amino hydrogens may be involved in the adsorption process, no conclusion may be drawn as to the exact nature of the adsorption mechanism.

Various effects have been utilized to explain the chromatographic behavior of some substituted anilines. Within the ortho-alkyl aniline series, the greatest effect appeared to be steric hindrance. Steric inhibition of resonance is not believed to be involved to any noticeable extent. It has been shown by the use of dipole moments that an N,N-dimethyl group is large enough to be twisted out of the aromatic plane by a methyl group, but that the primary amino group is too small to be thus disturbed. Molecular models show that even a t-butyl group does not prevent the amino group from remaining within the aromatic plane.

The other effects mentioned earlier might further be broadened to include hyperconjugation. As noted by Carlton and Bradbury (1), hyperconjugation has been proposed as accounting for the para-methyl group having a greater influence on the ionization of benzoic acids than the ethyl or isopropyl group. Sigma values given by Hammett (21) indicate that the order of influence of para-alkyl groups is t-butyl > methyl > ethyl = isopropyl. On the basis of inductive effects alone one would predict the order to be t-butyl > isopropyl > ethyl > methyl (22). Lichtin and Bartlett (23) observed the effect of 4-alkyl substitution on the ionization of triphenylmethyl chloride in liquid sulfur dioxide and estimated that the inductive and hyperconjugative effects of the t-butyl group were approximately the same, while the hyperconjugative effect of the methyl group was considerably higher than its inductive effect. A combination of the two effects, however, resulted in very nearly the same influence for the two groups on the ionization of the chloride. The combined hyperconjugative and inductive effects of all of the 4-alkyl groups had about the same influence on the chromatographic behavior of the phenols studied by Carlton and Bradbury (1). The same outcome is noted in this study of the 4-alkylanilines since their R values are the same within the limits of experimental error.

This study of the chromatographic behavior of various substituted anilines has demonstrated several points of a more practical nature for the practicing chemist. It has been well demonstrated that partially deactivated adsorbents can be used very effectively to separate ortho and para isomers. An easily performed chromatographic

procedure will reveal almost quantitative information as to the purity of an ortho or para substituted aniline with respect to the presence of the second isomer, and will probably give some indication of any meta compound present.

Two streak reagents have been evaluated and both were found to be useful for the detection of substituted anilines. p-Nitrobenzenediazonium tetrafluoroborate usually gives brighter colors and often gives sharper zones than ceric nitrate; however, the diazonium reagent fails to give a colored reaction product more frequently than does the ceric nitrate. The ceric nitrate failed to give a colored reaction product in only two cases, those of p-phenylenediamine and 2,4,6-tri-t-butylaniline. In both of these cases, the diazonium reagent did produce a color. The conjunctive use of these two reagents should detect most anilines.

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PART III

AN EVALUATION OF 2,4,7-TRINITROFLUORENONE AS A STREAK REAGENT

CHAPTER I

INTRODUCTION

With the proper streak reagents, one can frequently determine the class to which a given compound belongs. It would often be helpful to be able to determine more about the structure of the compound than simply the functional group present. A reagent which would distinguish between aliphatic and aromatic compounds, and the degree of nucleation in aromatic compounds, would be of real value in this connection. Two reagents of limited applicability have been reported by LeRosen, Moravek, and Carlton (1). Formaldehyde in sulfuric acid and antimony pentachloride in carbon tetrachloride have been reported by these authors as detection reagents for aromatic hydrocarbons. Further, the reagents generally distinguished between noncondensed rings and condensed nuclei by their color reactions.

The compound, 2,4,7-trinitrofluorenone, has been reported in the literature as a reagent for both polynuclear aromatic compounds and some substituted benzenes (2, 3, 4, 5). The reagent has been particularly successful for the preparation of derivatives of certain aromatic compounds by microscopic fusion analysis (4, 5). It was decided to evaluate its usefulness as a streak reagent in chromatography. If 2,4,7-trinitrofluorenone could be adapted to use on adsorption columns, it would be a particularly welcome addition to the list of reagents applicable to the identification of components of complex organic

mixtures after chromatographic separation. Best results would be obtained if there were a sharp color distinction between noncondensed rings and the various condensed ring systems.

The colors obtained with formaldehyde in sulfuric acid and antimony pentachloride in carbon tetrachloride span the spectrum and in some cases the colors are not different between condensed nuclei and nonfused rings. For example, the formaldehyde in sulfuric acid gave a tan color with mesitylene and a brown color with 2-naphthol. With antimony pentachloride, methylaniline gave a tan color and 2-naphthol gave a brown coloration. 2,4,7-Trinitrofluorenone produced a yellow coloration with 2-naphthol and a yellow coloration with biphenyl. The reagent gave colored reaction products with substituted benzenes only when one of the substituents was a phenyl group. The reagent did react with almost all of the polynuclear aromatic compounds tested.

CHAPTER II

CHEMICALS, EQUIPMENT, AND PROCEDURE

Chemicals

The following chemicals were obtained in as pure a form as possible and used as obtained: naphthalene, phenanthrene, β-naphthol, α-naphthylamine, β-naphthylamine, 1,4-naphthoquinone, anthracene, bromobenzene, chlorobenzene, nitrobenzene, benzoic acid, anisole, benzaldehyde, o-terphenyl, m-terphenyl, p-terphenyl, o-aminobiphenyl, benzidine, α-bromonaphthalene, α-chloronaphthalene, 6-chloroquinoline, 9,10-dibromoanthracene, 5,7-dibromo-8-hydroxyquinoline, biphenyl, dichloronaphthalene, fluorene, quinaldine, quinaldinic acid, quinoline, isoquinoline, xanthydrol, xanthone.

2,4,7-Trinitrofluorenone.--This compound was obtained in a purified form from Matheson, Coleman, and Bell. Saturated solutions were made up in glacial acetic acid and a solvent mixture of 10 ml. of anhydrous methanol and 2 ml. of benzene.

Silicic acid.--Silicic acid was prepared as in Part II of this thesis. The water content was 12.75 per cent by weight.

Florisil.--Florisil was prepared as in Part II of this thesis. The water content was 2.35 per cent by weight.

Petroleum ether.--Petroleum ether, Merck Reagent Grade, was used as obtained.

Equipment

The equipment used in this work was the same as that used in Part I of this thesis.

Procedure

The compounds were weighed into glass-stoppered Erlenmeyer flasks and benzene was added from a burette to make 0.01 M solutions. The procedure for the chromatography of these compounds was the same as that used in Part I of this thesis for the esters except that heat was not always used to bring out a coloration. The columns were streaked once with a 2,4,7-trinitrofluorenone solution, and the infrared lamp was used for heating if no coloration developed within two minutes. The columns were packed to a height of 75 ± 5 mm. At least duplicate columns were run for each compound.

CHAPTER III

RESULTS AND DISCUSSION

The results of the tests made with 2,4,7-trinitrofluorenone are listed in Tables 1 and 2. Blank spaces in the tables indicate that no reaction was observed with that compound. Table 1 contains the data obtained using silicic acid with 12.75 per cent water by weight, and Table 2 contains the data obtained using Florisil with 2.35 per cent water by weight. All of the compounds detected were either adsorbed at the top of the column or at the bottom of the column. R values for those compounds adsorbed at the top of the column are listed as 0.00 even though the front edge of the zone moved a short distance down the column.

The columns which gave no observable zone when streaked with a saturated solution of 2,4,7-trinitrofluorenone in the mixture of methanol and benzene were also streaked with the saturated solution of 2,4,7-trinitrofluorenone in glacial acetic acid. Orchin and his coworkers (3) have reported that 2,4,7-trinitrofluorenone addition compounds with low molecular weight derivatives of naphthalene separated more rapidly from glacial acetic acid. Neither of the solutions was found superior to the other in this work. The mixed methanol-benzene solvent was preferred since its vapors were less offensive.

The reagent did not react with many compounds other than those containing fused nuclei. With substituted benzenes, the reagent

Table 1. Results of 2,4,7-Trinitrofluorenone

Streak on Silicic Acid

Compound	Heating Time (minutes)	Zone Color	R Value
Naphthalene	3	Yellow	1.00
β-Naphthol	0	Yellow	1.00
α-Naphthylamine	-	--	--
β-Naphthylamine	3	Gray	0.00
1,4-Naphthoquinone	-	--	--
Anthracene	0	Yellow	1.00
Phenanthrene	0	Yellow	1.00
Bromobenzene	-	--	--
Chlorobenzene	-	--	--
Nitrobenzene	-	--	--
Benzoic Acid	-	--	--
Anisole	-	--	--
Benzaldehyde	-	--	--
o-Terphenyl	-	--	--
m-Terphenyl	-	--	--
p-Terphenyl	-	--	--
Aniline	-	--	--
o-Aminobiphenyl	-	--	--
Benzidine	0	Gray	0.00
α-Bromonaphthalene	3	Yellow	1.00

Table 1. (continued)

Compound	Heating Time (minutes)	Zone Color	R Value
α-Chloronaphthanene	3	Yellow	1.00
6-Chloroquinoline	-	--	--
9,10-Dibromoanthracene	0	Red	1.00
5,7-Dibromo-8-hydroxyquinoline	-	--	--
Biphenyl	3	Yellow	1.00
Dichloronaphthalene	3	Yellow	1.00
Fluorene	3	Yellow	1.00
Quinaldine	-	--	--
Quinaldinic Acid	-	--	--
Quinoline	-	--	--
Isoquinoline	-	--	--
Xanthydrol	-	--	--
Xanthone	-	--	--

Table 2. Results of 2,4,7-Trinitrofluorenone

Streak on Florisil

Compound	Heating Time (minutes)	Zone Color	R Value
Naphthalene	3	Yellow	1.00
β-Naphthol	3	Yellow	1.00
α-Naphthylamine	0	Gray	0.00
β-Naphthylamine	0	Gray	0.00
1,4-Naphthoquinone	-	--	--
Anthracene	0	Red	1.00
Phenanthrene	0	Yellow	1.00
Bromobenzene	-	--	--
Chlorobenzene	-	--	--
Nitrobenzene	-	--	--
Benzoic Acid	-	--	--
Anisole	-	--	--
Benzaldehyde	-	--	--
o-Terphenyl	3	Yellow	1.00
m-Terphenyl	3	Yellow	1.00
p-Terphenyl	3	Yellow	1.00
Aniline	-	--	--
o-Aminobiphenyl	3	Yellow	1.00
Benzidine	0	Gray	0.00
α-Bromonaphthalene	3	Yellow	1.00

Table 2. (continued)

Compound	Heating Time (minutes)	Zone Color	R Value
α-Chloronaphthalene	3	Yellow	1.00
6-Chloroquinoline	-	--	--
9,10-Dibromoanthracene	0	Red	1.00
5,7-Dibromo-8-hydroxyquinoline	3	Gray	0.00
Biphenyl	3	Yellow	1.00
Dichloronaphthalene	3	Yellow	1.00
Fluorene	3	Yellow	1.00
Quinaldine	-	--	--
Quinaldinic Acid	-	--	--
Quinoline	-	--	--
Isoquinoline	-	--	--
Xanthydrol	-	--	--
Xanthone	-	--	--

was useless under the conditions employed for this work except for those cases where the substituent was a phenyl or substituted phenyl group. With substituted quinolines, the reagent gave no color except in the case of 5,7-dibromo-8-hydroxyquinoline. The reagent gave better colors when used on Florisil than when used on silicic acid.

Solvents studied included petroleum ether, benzene, chloroform, n-butyl ether, and iso-octane. Some of the solvents would move those adsorptives which were adsorbed at the top of the column with petroleum ether developer. None of the developers studied would prevent the compounds not adsorbed using petroleum ether as developer from moving to the bottom of the column. Petroleum ether was used throughout the study as the developer.

Difficulty was experienced in preventing the tops of the columns from smearing on extrusion when petroleum ether was used as the developer. This tendency to smear was overcome by placing a wad of surgical cotton on top of the column when the solvent front reached the bottom of the column. The column was then extruded and the cotton wad was removed.

This evaluation of 2,4,7-trinitrofluorenone as a streak reagent has indicated that the compound may be useful in chromatograph in a limited manner. For example, it may be used conjunctively with formaldehyde in sulfuric acid and/or antimony pentachloride in carbon tetrachloride in the following manner: 2,4,7-Trinitrofluorenone gives a distinct red color with anthracene and its derivatives on Florisil, while the other two reagents give yellow-green or blue-green colors. This,

and other cases where the color is quite distinct, would make 2,4,7-trinitrofluorenone somewhat of a confirmation streak reagent when the color of the other two reagents is not distinct.

There is no general distinction between mono- and polynuclear aromatic compounds, although detection is distinct on Florisil columns. In those cases where heat was not required for the initial production of color, the color did become more intense upon the application of heat. This suggests that longer heating periods might bring out colored zones in more cases, but if the heating time is extended much past three minutes, the column begins to crumble and the outer surface separates into sections which tend to curl slightly at the edges. This result makes any observation of color or position of zone indeterminate.

2,4,7-Trinitrofluorenone is not evaluated as an extremely useful reagent. As noted above, it may resolve certain cases when its own colored product is unique in color. Otherwise, it tends to give yellow colors with sundry polynuclear aromatic compounds. Mononuclear aromatic compounds gave no color reaction unless one substituent was a phenyl group. This exclusion of many mononuclear compounds from detection by this reagent makes its use limited.

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VITA

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